BIO-BASED APPROACHES TO INORGANIC MATERIAL SYNTHESIS (POSTPRINT)

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Bio-based approaches to inorganic material synthesis

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Abstract
Nature is an exquisite designer of inorganic materials using biomolecules as templates. Diatoms create intricate silica wall structures with fine features using the protein family of silaffins as templates. Marine sponges create silica spicules also using proteins, termed silicateins. In recent years, our group and others have used biomolecules as templates for the deposition of inorganic materials. In contrast with the traditional materials science approach, which requires high heat, extreme pH and non-aqueous solutions, the bio-based approaches allow the reactions to proceed usually at near ambient conditions. Additionally, the biological templates allow for the control of the inorganic nanoparticle morphology. The use of peptides and biomolecules for templating and assembling inorganics will be discussed here.

Bio-inorganic materials in Nature

There are examples throughout Nature of organisms utilizing proteins and other biomolecules to create inorganic structures, such as bone, shell, diatom cell walls and sponge spicules. These structures generally are formed at ambient conditions and near neutral pH. Well-studied examples include the silaffin family of proteins from diatoms. These proteins have been shown to be involved in silica deposition in diatoms, and have been isolated from diatoms to show that they template silica deposition *in vitro* [1]. Similarly, a different family of proteins, the silicateins, has been found in marine sponges that template the formation of silica spicules [2]. Both of these protein families are being dissected to determine what roles the different members play in the deposition and shape of the resulting inorganic structures.

Another example of silica deposition in Nature comes from the interaction of amorphous silica in grasses with a plant pathogen. In this case, there is a biotransformation of naturally occurring, amorphous silica into crystalline silica nanoparticles. This process involves the fungus and plant pathogen *Fusarium oxysporum* acting on amorphous silica in rice husks to transform it into crystalline silica nanoparticles after 24 h at room temperature [3]. Specific cationic proteins from *F. oxysporum* were found to be associated with the crystalline silica after the reaction occurred. However, these cationic proteins alone did not induce crystalline silica formation from the amorphous silica precursor [3]. Clearly there must be other components from the fungus that play a role in templating or ordering the silica into the crystalline form.

Biotemplates for inorganic nanomaterials

Based on the findings from Nature, many groups have studied the use of naturally occurring proteins, domains of those proteins or analogous proteins (i.e. with similar pI or amino acid composition) to template inorganic materials. One successful approach has been the use of R5 peptide, which is the fifth repeating peptide unit of the silaffin polypeptide sil1p, to template both silica as well as titanium nanoparticles from their precursor solutions [4–6]. The studies with R5 have shown that this 19-amino-acid peptide is incorporated into the silica matrix as it is templated. This finding led to the successful co-encapsulation of enzymes and other nanoparticles into the porous silica matrix when R5 is used as the template [5]. When encapsulated, the enzymes are as active as in the free state but have increased stability against dehydration, heating and storage at room temperature [4,5]. By incorporating a magnetic nanoparticle into the silica matrix, the particles can be separated from the bulk solution simply by applying a magnetic field. This opens the door for multifunctional systems composed of an R5-templated silica matrix into which are incorporated both an enzyme and a magnetic nanoparticle [5]. This multifunctional system could be used for mobile decontamination of water supplies, for example, by encapsulating an enzyme that would neutralize the contaminant and then be removed from the bulk by applying a magnetic field to the solution.

For a number of years poly(L-lysine) has been known to precipitate silica [7–9]. However, more recently it has been found that different lengths of poly(L-lysine) result in different shapes of silica (Figure 1). Chains with fewer than 100 lysine residues induce spherical silica structures to form after reacting with the silicic acid precursor. However, with greater than 100 lysine residues, hexagonal silica platelets are formed [10]. The underlying mechanism of action of this dramatic change in morphology is based on a change in the secondary structure of poly(L-lysine) polypeptide from random coil
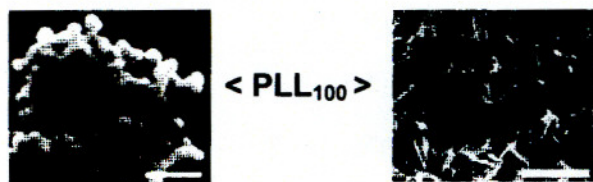
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Figure 1 | The effect of biopolymer chain length on silica morphology

Silica structures formed with different chain lengths of poly(L-lysine). Scale bar, 2 μm.

to α-helix in the presence of silicic acid with poly(L-lysine) with greater than 100 residues. The shorter chain lengths do not exhibit any structural transitions and give rise to spherical silica particles [10]. These results show that by modifying the biopolymer chain length, tuning of the resulting inorganic morphology is possible. This is one of the key advantages of using biomolecules as templates for inorganic synthesis: the biomolecules can be tailored specifically to achieve the desired nanostructure.

Another example of using a biotemplate to control morphology is in the case of ZnO synthesis. We have identified a peptide named ZnO1 from a phage peptide library that binds to ZnO nanoparticles. Addition of the ZnO1 peptide with zinc nitrate and hexamethyltetramine resulted in the formation of ZnO hexagonal platelets in solution (M.M. Tomczak and R.R. Naik, unpublished work). In contrast, the control sample without ZnO1 peptide contains mainly elongated hexagonal rods. These results indicate that the ZnO1 peptide inhibits growth along the c-axis face of the ZnO crystal. By exploiting interactions such as this, the ability to form nanostructures of desired shape and size using tailored biomolecules is possible.

More recently, peptides have been used in the synthesis of more complex materials. An example of this is the room temperature formation of calcium molybdate (CaMoO₄) using a peptide termed CM4, which was identified through phage display [11]. The findings show that the resulting CaMoO₄ is crystalline and photoluminescent. This is the first literature report of a peptide that templates a crystalline multicomponent oxide at ambient conditions. Crystalline multicomponent oxides are important components of functional devices and their room temperature synthesis using a method such as that described by Ahmad et al. [11] potentially decreases both the cost and time involved in synthesis.

Peptide-mediated synthesis of gold

Peptides represent a significant component in gold nanoparticle synthesis given their ability to control all aspects of synthesis including the reduction of gold salt precursor [12–16], while also providing a highly active surface for biorecognition, catalysis and assembly [16,17]. Recently, it has been demonstrated that tyrosine plays a fundamental role in the reduction of Au^{3+} to Au^{0} by a number of tyrosine-containing peptides, but most are limited to simple dipeptides and tripeptides [12–16]. Unfortunately, each peptide produces gold nanoparticles of various sizes, morphology and irregularity, and, as a result, there is little correlation between sequence and properties beyond simply containing a tyrosine residue.

The A3 peptide, identified through phage display, and found to contain a positionally isolated tyrosine residue, exhibits good gold reduction activity and forms nearly monodisperse particles stable in solution for months [16]. Consequently, given its propensity for gold formation, A3 represents a good model sequence to examine the effects of amino acid substitutions on gold synthesis and as a means to tailor nanoparticle characteristics. We examined five different variants of the A3 peptide which addressed substitution, deletion, rearrangement and scrambling of key residues within the sequence. More specifically, these involved the substitution of tyrosine with a serine residue (A3-S), the exchange of tyrosine and methionine residues (A3-X), replacement of methionine with an alanine (A3-A), replacement of serines with prolines (A3-P) and scrambling of the sequence (A3-W) (Table 1). These peptide variants were then tested for gold nanoparticle binding affinity, gold reduction activity, nanoparticle size and dispersity. The peptide variants were screened for binding against gold nanoparticles using a fluorescence-based assay. Analysis of gold binding showed that the A3-A peptide (alanine-substituted) exhibited the largest affinity for gold, while A3-P (proline-substituted) showed almost no binding. This suggests that replacement of methionine with an alanine might enhance nanoparticle binding, but the removal of serine residues severely reduces affinity of the peptide. The remaining peptides (A3-S, A3-X and A3-W) revealed moderate binding to gold, in addition to possessing equal binding affinities across the group regardless of the modification (Table 1). Based on these results, it appears that the hydroxy groups are required for strong binding to the gold nanoparticle surface. This observation is further supported by the work of Sarikaya and co-workers [18] who have demonstrated that peptides that contain serine and threonine bind more tightly to gold surfaces.

In addition to nanoparticle binding, the mutant peptides rapidly reduced Au^{3+} to Au^{0}. Upon addition of Au^{3+}, the solution immediately turned red in appearance and yielded a plasmon resonance characteristic of gold by UV–visual spectroscopy with the exception of the tyrosine-deleted A3-S peptide. This is consistent with gold reduction by other tyrosine-containing templates [14,19]. The resulting nanoparticles with the mutant peptides showed slightly shifted plasmon resonance peaks from the parent A3 peptide (Table 1), but an overall similarity among the spectra. In contrast, nanoparticles synthesized using the A3-W peptide (scrambled sequence) had a broadened plasmon peak shifted to 538 nm and featured a shoulder at approx. 650 nm (Figure 2).

The peptide-synthesized nanoparticles were characterized by TEM (transmission electron microscopy) and sedimentation on a sucrose gradient using a CPS (centrifuge particle size) disc analyser. CPS analysis serves as a complementary characterization tool to TEM by providing a statistical

Table 1 | Nanoparticle properties with modified A3 peptides

NA, not applicable; NP, nanoparticle.

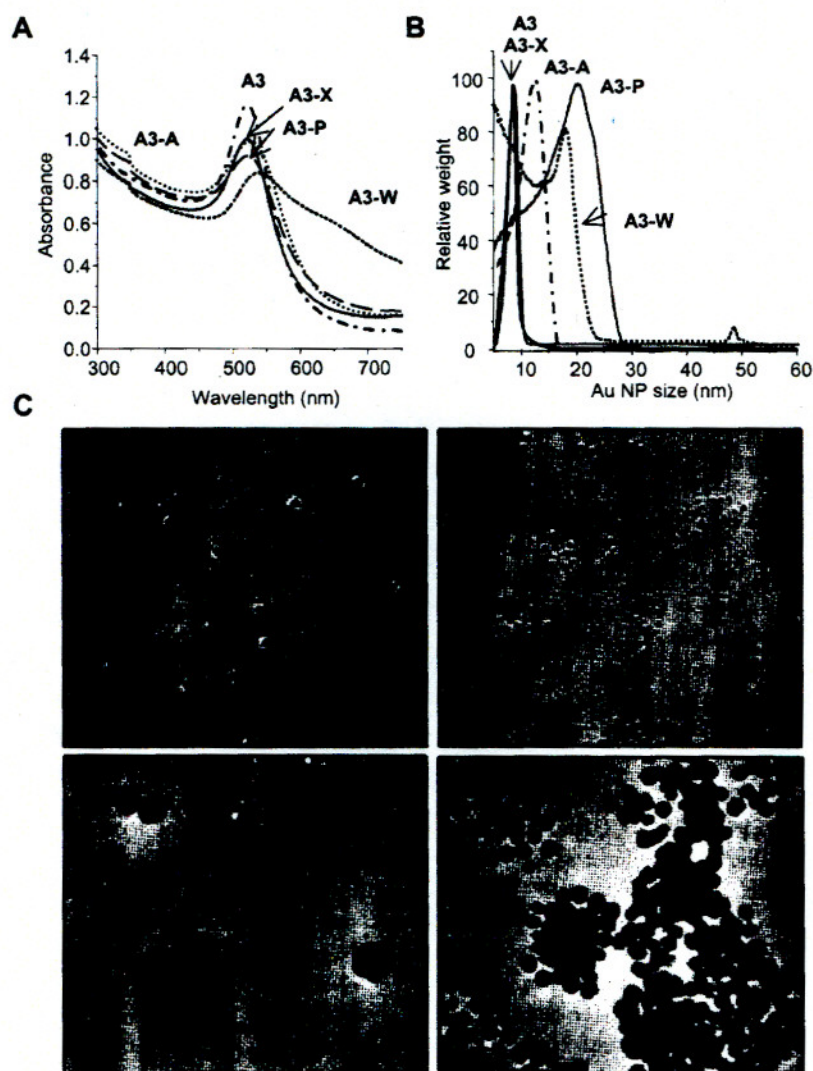| Au-peptide NP	Sequence	Au NP binding (%)*	Absorbance (nm)	TEM Au NP size (nm)	CPS Au NP† size (nm)
A3	AYSSGAPPMPPF	–‡	523	12.8 ± 2.9	8.6 ± 0.8
A3-S	ASSSGAPPMPPF	NA	NA	NA	NA
A3-X	AMSSGAPPYPPF	9.6 ± 3.5	521	12.9 ± 2.8	8.4 ± 1.0
A3-A	AYSSGAPPAPPF	16.1 ± 3.8	527	8.3 ± 3.6	12.6 ± 1.9
A3-P	AYPPGAPPMPPF	1.6 ± 1.0	522	9.1 ± 6.4	20.4 ± 5.1
A3-W	PSPGSAYAPFPM	9.9 ± 3.9	538	13.6 ± 3.1	18.9 ± 3.3

*Percentage binding was determined by difference in fluorescence of tyrosine ($\lambda_{ex} = 270$ nm/$\lambda_{em} = 303$ nm) with three different samples. Peptide was incubated with 30 nm gold colloids (sigma) for 2 h.

†Sizes were determined using a CPS disc analyser and calibrated for each sample using a 377 nm PVC [poly(vinyl chloride)] standard.

‡Percentage binding was indeterminate due to low peptide concentration used for A3.

Figure 2 | Biosynthesis of gold nanoparticles using peptides

(A) UV-visual spectra of gold nanoparticles synthesized using the various peptides and (B) the size distribution based on centrifugal particle sedimentation analysis. (C) TEM micrographs of gold nanoparticles synthesized using peptides.

comparison of the total size population of nanoparticles. CPS analysis revealed that nanoparticles produced with the A3-X peptide had a monomodal particle size distribution equivalent to the parent A3 sequence, whereas the remaining mutant peptides yielded nanoparticles with significantly larger sizes, dispersities and multimodal distributions (A3-W) (Figure 2). Consistent with CPS results, the TEM micrographs showed a narrow distribution of sizes for gold nanoparticles from the parent A3 and mutant A3-X peptides (Table 1). Although beyond these similarities, A3-X nanoparticles differ from the parent A3 particles by their propensity to assemble on a TEM grid. For example, nanoparticles synthesized from the parent A3 sequence exhibited a moderate degree of ordering and packing, while the A3-X particles were not inclined to assemble. Switching the position of tyrosine and methionine respectively within the base sequence does not affect nanoparticle characteristics, but invariably changes the properties of the peptide coat on gold with regard to ordering of nanoparticles on a substrate. Alternatively, the single mutation of methionine to alanine (A3-A), which featured a high affinity for gold, yielded the smallest sized nanoparticles by TEM, while the double proline substitution resulted in the largest distribution of sizes and presence of non-spheroidal particles. In the TEM micrograph showing the nanoparticles synthesized using the A3-P peptide, ~11% of the particles contained facets and various shapes (Figure 2). Similar shape control has also been observed using biomolecules to accelerate the nucleation of particles along a preferred direction [20,21].

Based on the results obtained using the peptide variants, the following observations can be made. (i) Polar amino acids such as serine are required for tight binding to the gold surface, (ii) polar amino acids might play a role in morphology control, and (iii) amino acid sequence context is also an important consideration.

Conclusions

These peptides represent only a fraction of the peptide sequences identified to date that bind to or can be used in the synthesis of inorganic nanomaterials. The bio-based synthesis of nanoparticles provides a promising route for fabricating complex hybrid materials. We have recently demonstrated the use of peptides to synthesize hybrid nanoparticles for use in catalytic applications [17]. Besides interest in the synthesis behaviour, the modified peptide coat also provides a wealth of surface functionalities that can be exploited for assembling and coupling nanomaterials to surfaces, polymers, and also can be tailored for sensing applications.

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